Proof of concept study for different-sized chitosan nanoparticles as carbon dioxide (CO₂) indicators in food quality monitoring

Seokjin Suh, Xiangpeng Meng, Sanghoon Ko*

Department of Food Science and Technology, Sejong University, 209 Neungdong-ro, Gwangjin-gu, Seoul 05006, Republic of Korea

A R T I C L E   I N F O

Article history:
Received 24 July 2016
Received in revised form 16 August 2016
Accepted 16 August 2016
Available online 17 August 2016

Keywords:
Chitosan
Nanoparticle
CO₂ indicator
Size control
Transition appearance time

A B S T R A C T

The objective of this study was to develop different-sized chitosan nanoparticles as CO₂-based food quality indicators. Chitosan nanoparticles were fabricated with different sizes (small, 300 nm; medium, 500 nm; and large, 1000 nm) by ionic gelation. To investigate the performance of chitosan nanoparticles as CO₂ indicators, they were suspended in aqueous solution at pH 8.0. Changes in the pH and absorbance of the suspension were measured over time, the absorbance at the transition appearance time being calculated using the inverse-Hill function. The resultant transition appearance times were 11.23, 14.33, and 27.69 min for the small, medium, and large-sized chitosan nanoparticles, respectively. Controlling the chitosan nanoparticle size enables the transition appearance time of the CO₂ indicator to be adjusted in order to match the change in quality of packaged food. This study suggests that different-sized chitosan nanoparticle-based CO₂ indicators can be used as food quality indicators.

© 2016 Elsevier B.V. All rights reserved.

Contents

1. Introduction ................................................................. 265
2. Materials and methods .................................................. 266
  2.1. Materials ............................................................. 266
  2.2. Fabrication of different-sized CNPs ................................. 266
  2.3. Measurement of particle size and zeta-potential of different-sized CNPs ........................................ 266
  2.4. Performance of different-sized CNP suspensions as CO₂ indicator ................................................. 266
  2.5. Determination of transition appearance time of size-different CNP-based suspensions as CO₂ indicator ........................................... 266
3. Results and discussion .................................................... 267
  3.1. Mean diameter of different-sized CNPs ............................... 267
  3.2. Zeta potentials of different-sized CNPs ................................ 267
  3.3. pH change in different-sized CNP-based CO₂ indicators ................................................................. 267
  3.4. Initial appearance of different-sized CNP-based CO₂ indicators .......................................................... 267
  3.5. Transition appearance in different-sized CNP-based suspensions as CO₂ indicator ........................................... 267
4. Conclusions ................................................................. 269
Acknowledgments ............................................................. 270
References ................................................................. 270

1. Introduction

Fermented foods are distributed to consumers in sealed packages in which persistent ripening by microorganisms, such as yeast and lactic acid bacteria, causes the production and accumulation of carbon dioxide (CO₂) in the package headspace [1]. However, it is difficult for consumers to determine the degree to which fermented foods have ripened in the package, and ongoing fermentation in food packaging limits the storage and distribution of fermented food products [2,3]. Therefore, a visual indicator corresponding to food quality, such as the degree of ripening, could be an easy method to allow consumers to determine...
whether fermented food is well ripened [4,5].

Recently, chitosan has been used as a CO2 indicator material in several studies. Appearance (turbidity) changes in chitosan suspensions depend on pH, and CO2 gas dissolves to form H2CO3, giving an acidic suspension. In the food package, the chitosan suspension is opaque at low CO2 concentrations (early ripening), but becomes transparent at high CO2 concentrations due to its solubility in acid [6,7]. This offers a way to visually identify the degree of ripening and quality of the fermented food, resulting in continuous attempts to use chitosan as a food quality indicator [8]. However, the performance of chitosan-based CO2 indicators is currently limited because CO2 emission characteristics, such as rate and quantity, depend on the type of food in the package. Therefore, it is important to develop indicator technology that is more sensitive to CO2 in the headspace of packaged foods.

Based on the principle that different-sized chitosan nanoparticles (CNPs) have different surface areas and physical sizes, we hypothesized that altering these properties could overcome the aforementioned problems by reacting sensitively with various fermented foods. The ripening status of various fermented foods can be monitored using CNPs with different sizes and surface areas, which have different solubilities in acidic solution at the same CO2 concentrations.

CNP size control is a key to developing a high-performance food quality indicator. The ionic gelation of chitosan forms inter- and intra-molecular linkages between the positively charged amine groups of chitosan and negatively charged polyanions such as tripolyphosphate (TPP) [9]. Previous studies have shown that the factors affecting nanoparticles formation depend on the concentration and molecular weight of chitosan, the degree of deacetylation, and the chitosan to TPP ratio [10,11]. Larger chitosan to TPP ratio forms bigger nanoparticles [12,13]. Increasing the chitosan to TPP ratio increased the chances of electrostatic and hydrophobic interactions because chitosan-chitosan and chitosan-TPP meet each other more frequently. Herein, we hypothesize that the control of balanced electrostatic and hydrophobic interactions between chitosan molecules and TPP is important for preparing nanoparticles with controlled sizes. In addition, the optimization of manufacturing procedures, including chitosan and TPP contents, is required to control electrostatic and hydrophobic interactions during ionic gelation. In this study, we attempted to fabricate chitosan with controlled sizes (small, 300 nm; medium, 500 nm; and large, 1000 nm) by manipulating certain process parameters, such as chitosan concentration and the chitosan to TPP weight ratio.

In order to develop different-sized CNP-based CO2 indicators for applications in various fermented foods, it was important to determine the accurate indicating performance of tailored CO2 indicators. Using a suitable mathematical model enables easy and accurate calculation of indicating properties of these CO2 indicators. In most cases, the optimal mathematical model, which introduces nonlinearity, is the sigmoidal model [14]. In this study, as a kind of sigmoidal model, the inverse-Hill function was used to evaluate the different-sized CNP-based CO2 indicators developed.

The objective of the current study is to develop different-sized CNP-based CO2 indicators with indicating performances tailored to the CO2 concentration in the headspace of the packaged food.

2. Materials and methods

2.1. Materials

Chitosan (molecular weight 22 kDa, degree of deacetylation 84%) was supplied by Kittolife Co. (Pyongtaek, Korea). Analytical grade sodium tripolyphosphate was supplied by Sigma Aldrich (St. Louis, MO, USA). Hydrochloric acid, acetic acid, sodium hydroxide, all of analytical grade, were purchased from Daejung Chemicals & Metals (Shiheung, Korea).

2.2. Fabrication of different-sized CNPs

CNPs were prepared by ionic gelation between the chitosan molecules and TPP. Briefly, 0.23% chitosan powder was dissolved in 1% (v/v) acetic acid to produce 300 nm-sized CNPs. The 500 and 1000 nm-sized CNPs were prepared using 0.30% and 0.50% chitosan powder, respectively, in the 1% acetic acid solution. TPP was dissolved separately in distilled water to a concentration of 0.7 mg/mL. Under magnetic stirring at 500 rpm, the TPP solution was added drop wise to the aqueous chitosan solution in a volume ratio of 3:1 using a 50 mL burette. The CNPs formed by self-assembled ionic gelation. The mixture was stirred at 500 rpm and 25 °C for 12 h.

2.3. Measurement of particle size and zeta-potential of different-sized CNPs

The size distribution and zeta-potential of the CNPs were measured using a commercial zeta-potential and particle size analyzer (DelsaNano C, Beckman Coulter, Inc., Fullerton, CA, USA) with an autotitrator. CNP particle sizes were measured by sampling 3 mL of the aqueous acetic acid solution. For zeta-potential measurement, CNPs in aqueous acetic acid were injected into the zeta-potential analyzer. The zeta-potential was measured at different pH values between 2 and 11. Both size and zeta-potential measurements were repeated three times for each sample.

2.4. Performance of different-sized CNP suspensions as CO2 indicator

To investigate the effect of the different-sized CNP-based suspensions as CO2 indicators, the initial pH of the CNP-based suspension was adjusted to 8.0 (opaque state) using 4.0 M NaOH solution, and then 100% CO2 gas was directly injected into the suspensions using a small needle as an accelerated test for 28 min.

The absorbance of different-sized CNP-based CO2 indicators was observed at 600 nm using a spectrophotometer (DU 730, Beckman Coulter Inc., Fullerton, CA, USA), while their pH was measured using an SP-2100 Laboratory pH/ORP Meter (Suntex Instruments Co., Ltd., New Taipei, Taiwan).

2.5. Determination of transition appearance time of size-different CNP-based suspensions as CO2 indicator

In order to determine the indicating properties of different-sized CNP-based CO2 indicators, the changing tendency of absorbance was analyzed numerically using a mathematical model. In this study, the inverse-Hill function was selected as the mathematical model to calculate various properties the CO2 indicators.

The inverse-Hill function is denoted as

\[ y = y_0 - \frac{a x^n}{b^n + x^n} \]

where \( x \) (time) and \( y \) (CO2 indicator absorbance) are the independent and dependent variables, respectively, \( y_0 \) (initial absorbance) is the y-intercept, \( a \) is the amplitude of the plot, and \( y_0 - 1 \) is the final absorbance of the indicator at low absorbance. The exponent \( n \) represents the steepness of absorbance changes, showing how slowly or rapidly the absorbance of different-sized CNP-based CO2 indicators decreases with CO2 concentration. At time \( b \), \( y \) is an approximate value of the half-amplitude of the plot. In other words, \( b \) is the time showing the most rapid change in absorbance during CO2 gas dissolution. In all different-sized CNP-
based CO₂ indicators, the change in appearance from opaque to transparent was easily observed with the naked eye at absorbance 0.30, obtained by preliminary experiments. The time to reach absorbance 0.30 was named as the transition appearance time and was calculated by reversing the equations obtained from the inverse-Hill functions for each different-sized CNP-based CO₂ indicator.

3. Results and discussion

3.1. Mean diameter of different-sized CNPs

CNPs were prepared by the self-assembled ionic gelation of chitosan molecules with TPP, a polyanion, involving reversible crosslinking with cationic chitosan through electrostatic interactions. Table 1 shows the effect of chitosan concentration on the mean CNP diameter. The mean diameters were 321.8 ± 29.6, 515.0 ± 120.0, and 1194.9 ± 375.0 nm for the 300, 500, and 1000 nm CNPs, respectively. To prepare the target 300 nm chitosan particles, a 0.23% chitosan aqueous solution was formulated. The 500 nm-sized chitosan particles were prepared from 0.30% chitosan solution and 1000 nm-sized particles were prepared from 0.50% chitosan solution. The as-formed CNPs tended to increase in size with increasing chitosan concentration [12,15]. The increase in CNP size could be attributed to the dense spatial distances between chitosan molecules at higher concentrations, resulting in larger particles forming (Fig. 3(C)). In contrast, smaller particle sizes were obtained at lower chitosan concentrations, which decreased viscosity during ionic gelation. Lower chitosan concentrations provided well-dispersed chitosan molecules, allowing efficient electrostatic interactions between cationic chitosan and anionic TPP (Fig. 3(A)).

The polydispersity index increased with increasing CNP size due to the particle size distribution range becoming broader as mean CNP size increased, in agreement with previous reports [16,17]. Fabricating CNPs with narrow size distributions is recommended, as it affects the steepness of the change in absorbance for CNP-based CO₂ indicators. In this study, the polydispersity of the CNPs prepared correlated with the exponent of absorbance (n) vs. time plot of CO₂ indicators with changing CO₂ concentrations. When exponent n was high, the transition in visual appearance of the CNP-based CO₂ indicators was easily determined. In future research, it would be important to control the polydispersity index within a certain range, because the performance quality of the CNP indicators is critically dependent on it.

3.2. Zeta potentials of different-sized CNPs

Fig. 1 shows the zeta-potentials of different-sized CNPs at different pH values. In general, the zeta-potential decreased with increasing pH value and the particles were positively charged under acidic and neutral conditions. The zeta-potentials of the different-sized CNPs ranged from approximate +10 to +50 mV under acidic conditions. Especially, all CNPs showed high zeta-potential values in the pH range 3–4. Regardless of CNP size, an increase in pH to basic conditions caused the zeta-potential to decrease to near-zero or negative values; for example, the zeta-potential of all CNPs at pH 8.0 was approximately zero. The amine groups confer a positive charge on the chitosan molecules [18,19]. Under acidic conditions, the positive charge increases due to the ionization of amine groups.

In order to utilize the different-sized CNP-based suspensions as CO₂ indicators, their initial appearance was set to opaque. The pH of the CNP-based suspensions was adjusted to 8.0, with zeta-potential of zero, which resulted in aggregation among the CNPs. As the pH decreased due to dissolved CO₂ in the CNP-based suspension, the chitosan aggregates were broken down into individual CNPs, turning the suspension transparent.

3.3. pH change in different-sized CNP-based CO₂ indicators

The pH changes in different-sized CNP-based CO₂ indicators are shown in Fig. 2(A). For all CNP sizes, pH decreased rapidly for an initial 6 min, gradually decreasing to almost 6.0 over 18 min, and remaining constant thereafter. The 1000 nm-sized CNP-based CO₂ indicator had a slightly higher pH value than the 300 and 500 nm-sized CNP-based CO₂ indicators after 18 min, attributed to the higher concentration of chitosan used to formulate the CNPs and the formation of larger aggregates. As mentioned earlier, 0.23, 0.30, and 0.50% chitosan contents were used to form 300, 500, and 1000 nm-sized CNP-based CO₂ indicator suspensions, respectively. Water molecules become trapped in inter-molecular linkages between positively charged amine groups in chitosan and negatively charged phosphate groups in TPP. Generally, chitosan molecules in acidic aqueous solution are positively charged and have a strong affinity for water molecules [20]. The water molecules trapped among the chitosan particles are not able to dissolve CO₂ molecules because they are bound and not free water molecules. In addition, forming larger aggregates by adjusting the pH of the 1000 nm-sized CNP-based CO₂ indicator increased the amount of water trapped inside the chitosan particles. Chitosan particles trap a large amount of water molecules in inter-molecular linkages because of their high water-holding capacity.

Free water molecules in the suspension contribute to dissolving CO₂, whereas those inside the chitosan particles cannot participate in dissolving CO₂ molecules. The CNP-based CO₂ indicator suspension with high chitosan concentration facilitated a relatively large amount of water molecules to be trapped compared with low chitosan concentrations. As a result, in the 1000 nm-sized CNP-based CO₂ indicator suspension, free water content decreased, while bound water content increased, indicating a slightly higher pH than those of 300 and 500 nm-sized CNP-based CO₂ indicators. The pH change in CNP-based CO₂ indicator suspension over time was essential for tailoring its indicating performance depending upon CO₂. The pH profiles of 300 and 500 nm-sized CNP-based CO₂ indicators were similar because the chitosan contents used to prepare them were quite similar (0.23% and 0.30% chitosan contents for 300 and 500 nm-sized CNPs, respectively). These results were in close agreement with those of a previous study [21]. This transition in visual appearance was dependent on the pH change in the CNP-based CO₂ indicators, since CO₂ dissolution behavior depends on the mean CNP size.

3.4. Initial appearance of different-sized CNP-based CO₂ indicators

Fig. 2(B) shows the change in absorbance for the CNP-based CO₂ indicators at pH 8.0. Initially, the absorbance values were 0.496, 0.710 and 1.381 for the small, medium, and large-sized CNP-based CO₂ indicators. The primary size of the CNPs affected the degree of light scattering in the suspensions, which increased with increasing particle size. In addition, inter-particle aggregation
among CNPs also affected light scattering in the suspension. The larger the primary particle or inter-particle aggregate, the more turbid the appearance of the CO\(_2\) indicator suspension [20].

In order to analyze changes in absorbance behavior for the CNP-based CO\(_2\) indicators, the inverse-Hill function was used as a non-linear regression model. The inverse-Hill function fitted well with explaining the relationship between absorbance and time for the different-sized CNP-based CO\(_2\) indicators (R\(^2\) = 0.95). The inverse-Hill function coefficients (\(a\), \(n\), \(b\), and \(y_0\)) of the CNP-based CO\(_2\) indicators are listed in Table 2.

The initial absorbance (\(y_0\)) represents the physical properties of aggregated CNPs, such as size and opacity. The amplitude (\(a\)) refers to the total change in absorbance for the CO\(_2\) indicator suspensions during CO\(_2\) injection. In this experiment, the initial absorbance (\(y_0\)) increased with increasing CNP size: 1000 nm-sized indicators had the largest \(a\) and \(y_0\) values, whereas 300 nm-sized indicators had the smallest \(a\) and \(y_0\) values. The \(y_0\) value of the CNP-based CO\(_2\) indicators was dependent on the chitosan concentration and the size of the primary CNPs and their aggregates. Especially, the sizes of primary particles and inter-particle aggregates were key factors in determining \(y_0\) for CO\(_2\) indicator suspensions. As shown in Fig. 3, the aggregation of 1000 nm-sized CNPs formed large particulates due to strong van der Waals force, whereas 300 and 500 nm-sized CNPs formed small and medium-sized particulates. As a result, the size of aggregated CNPs affected \(y_0\).

The inter-particle aggregation among CNPs was explained by classic Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, which determines the stability of a suspension from attractive van der Waals forces and repulsive forces. Under acidic aqueous conditions, there was a large repulsive force relative to the attractive van der Waals force among the CNPs due to relatively high positive charges on the amino groups in chitosan. Conversely, under neutral and weak basic aqueous conditions, chitosan is not charged and van der Waals forces give rise to inter-particle aggregation, resulting in larger chitosan particles [19].

3.5. Transition appearance in different-sized CNP–based suspensions as CO\(_2\) indicator

As CO\(_2\) was injected into the different-sized CNP-based CO\(_2\) indicator suspensions, their opacity was almost maintained for the initial 6 min. After 6 min, the absorbance of the CNP-based CO\(_2\) indicators began to decrease over time. At 6 min, the CNP-based CO\(_2\) indicators were at pH 6.5, at which inter-particular aggregates start to be break down into individual CNPs. Thereafter, as the CNP-based suspensions became more acidic, the more transparent their appearances became (Fig. 3). As a result, chitosan suspensions under neutral and weak basic aqueous conditions were opaque, but turned transparent on becoming acidic. As CO\(_2\) was injected over time, 300 and 1000 nm-sized CNP-based indicators showed the slowest and fastest reductions in absorbance, respectively, considered to result from their different surface areas. The larger surface areas of the 1000 nm-sized CNPs resulted in more frequent contact with the acidic solution, resulting in the rapid decomposition of inter-particular aggregates. As time elapsed, the absorbance of the CNP-based CO\(_2\) indicators decreased steadily to almost 0.25, with the time-frame required to reach this absorbance observed to be delayed by increasing CNP size, in agreement with the results mentioned above.

The appropriate rate of change for absorbance under CO\(_2\) injection was key to controlling the performance of the transition appearance in CNP-based CO\(_2\) indicators. Among the inverse-Hill
sorbance occurred, and was an important factor for evaluating the performance of CO2 indicators. The $b$ value represents the inflection point, i.e., the most rapid change in absorbance is observed at $b$. In this experiment, 300, 500 and 1000 nm-sized CNP-based CO2 indicators gave $b$ values of 8.08, 7.86, and 12.45 min, respectively. The $b$ value was delayed with increasing CNP size in the suspensions. The 1000 nm-sized CNPs and their aggregates contained larger chitosan content than the 300 and 500 nm-sized CNPs. The most rapid change in absorbance appeared later in the 1000 nm-sized CNP suspension, even though the change in rate of absorbance ($n$) was the fastest. Therefore, the $b$ value was delayed with increasing CNP size. This transition behavior was related to the chitosan concentration in the CO2 indicator suspension and the surface area of the aggregated CNPs. The $b$ value of different-sized CNP-based CO2 indicators mutually correlated with $a$, $y_0$, and $n$, because $a$ and $y_0$ were affected by the chitosan concentration used in suspension preparation, and $n$ was affected by the surface area of aggregated CNPs. There was no difference between the $b$ values of 300 and 500 nm-sized CNP-based CO2 indicators despite the different numerical values of $a$, $n$, and $y_0$. It seems that complementary effects between $n$ and $y_0$ resulted in similar $b$ values for 300 and 500 nm-sized CNP-based CO2 indicators.

The indicating performance of CNP-based CO2 indicators was described well using the $b$ values, which is difficult to determine accurately with the naked eye. The $b$ value is not the visually transparent absorbance, but is simply the approximate value of the half amplitude of the plot. Instead, a visually identifiable point, which can be easily used to indicate food quality to consumers, is more effective for use in CO2 indicators. For that reason, we selected a criterion for the absorbance value of 0.30, which was determined by preliminary experiments. Thus, the time to reach the absorbance is called as ‘transition appearance time’, which is the most important point for evaluating the performance of the CO2 indicators studied herein. Similar to other coefficients, such as $a$ and $y_0$, the transition appearance time increased with increasing CNP size. The 300, 500, and 1000 nm-sized CNP-based CO2 indicators had transition appearance times of 11.23, 14.33, and 27.69 min, respectively (Table 3). In particular, 1000 nm-sized CNP-based CO2 indicator showed the latest transition appearance time, which was approximately twice later than those of the 300 and 500 nm-sized CNP-based CO2 indicators. The transition appearance time of the 500 nm-sized CNP indicator appeared after 3.10 min, compared with the 300 nm-sized CNP-based CO2 indicator. At the transition appearance time, the pH of all the indicators was roughly 6.0 and the appearance was not completely transparent, indicating that the transition from pH 8.0–6.0 broke down some aggregates into individual chitosan nanoparticles, and all different-sized CNP-based CO2 indicators were not fully reversible in this experiment, in agreement with the literature [19]. The significant differences in transition appearance time showed the potential of these CNP-based CO2 indicators to have tailored indicating performance for various food applications. In particular, these CNP-based CO2 indicators could be selectively applied to fermented foods as food quality indicators.

4. Conclusions

CNPs with different sizes (300, 500, and 1000 nm) have the potential to be applied as tailored CO2 indicators for different food types and amounts in packaging. The indicating performance, such as the rate of absorbance, could be tailored by applying different-sized CNPs to CO2 monitoring. Especially, the transition appearance time was delayed by increasing CNP sizes, meaning that small CNP-based CO2 indicators could be used for monitoring foods emitting small amounts of CO2 gas during fermentation, and so on. The size control of CNP-based CO2 indicators enables their
application to various fermented foods with different ripening behaviors. In conclusion, different-sized CNP-based CO2 indicators can effectively reflect changes in CO2 concentration at different rates and be used to monitor the quality of fermented foods with various CO2 emission characteristics.

Acknowledgments

This research was supported by the Agriculture Research Center (ARC, 710003) program of the Ministry of Agriculture, Food, and Rural Affairs, Republic of Korea.

References